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Nicotine Treatment for Pulmonary Sarcoidosis: A Clinical Trial Pilot Study

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A. Introduction

Sarcoidosis is a systemic granulomatous disease of unknown cause, most commonly affecting the lungs, afflicting young to middle-aged adults in the prime of their lives, for which highly effective and well-tolerated therapies are lacking. First-line therapies for sarcoidosis are often ineffective, poorly tolerated and promote long-term health complications. Industry-sponsored clinical trials have tested proprietary, expensive and potentially toxic therapies that are reserved for refractory cases of sarcoidosis (1). Given that nicotine patches are FDA approved for other indications and readily available to the public without a prescription, it is unlikely that a multi-center placebo-controlled nicotine trial for sarcoidosis will ever be conducted without NIH-support. *In keeping with the NIH's goal to **"repurpose"** existing biologics, the proposed Clinical Trial Pilot Study will provide preliminary data required to design subsequent Phase II/III trials to evaluate nicotine as a novel low-cost, highly-effective, and safe treatment option for patients with active pulmonary sarcoidosis.*

Nicotine may be beneficial for the treatment of pulmonary sarcoidosis. At least three independent epidemiological studies indicate that smokers and chewing tobacco users, chronically exposed to nicotine, have a ~2-fold lower risk of developing sarcoidosis (2-4). Nicotine reprograms inflammatory pathways through the actions of α7 nicotinic receptors (5), thereby inhibiting various pro-inflammatory immune responses (5,6), particularly prototypical Th1 immune responses (7,8), which are the hallmark of sarcoidosis and other granulomatous disorders. Reprogramming of Th1 immunity could explain the benefits of nicotine treatment for Crohn's disease (9) and hypersensitivity pneumonitis (10), granulomatous disorders of the intestines and lungs, respectively. Our pilot study showed that nicotine is well-tolerated, and normalizes Th1 type immune responses in patients with active pulmonary sarcoidosis (11). The *specific objective* of this project will determine if nicotine treatment is safe and efficacious for patients with active pulmonary disease despite conventional therapy.

B. Background

Sarcoidosis is characterized by the development of non-necrotizing granulomatous inflammation in the absence of identifiable infectious or environmental causes. The disease typically involves the lungs, frequently leading to impaired exercise tolerance and associated dyspnea. A majority of patients with active pulmonary sarcoidosis complain of overwhelming fatigue, which often persists despite administration of immune-modulating drugs typically used to treat sarcoidosis (12,13). Furthermore, conventional treatments are associated with a spectrum of serious untoward effects, including diabetes mellitus, osteoporosis, bone marrow suppression, severe infections, cirrhosis, etc. (14,15). Indeed, the use of corticosteroids, the mainstay of pulmonary sarcoidosis treatment, is independently associated with a reduced quality-of-life (16). Although potent anti-TNFα drugs can be effective alternatives to usual care (1,14,15) they are exceedingly expensive, many insurance agencies (e.g., Medicare) and the FDA do not approve their use for sarcoidosis, and they predispose to serious complications, including the reactivation of latent TB, serum sickness, and increased risk of malignancy (17). As such, these agents are typically reserved for the treatment of refractory disease. Nicotine, through its actions on α7-nicotinic receptors, possesses potent immune-modulating actions. At higher concentrations (μM), nicotine suppresses antigen-mediated TNFα production (5,6), induces regulatory T cells (18-20), suppresses Th1 type immune responses (7,8), particularly in the lung (21,22), and, as such, is expected to suppress Th1-dependent granuloma formation. In support of a benefit in sarcoidosis, large epidemiological studies consistently show that chronic nicotine use (smoking or smokeless tobacco) reduces the risk of developing sarcoidosis (2-4). Moreover, by stimulating the dopaminergic axis of the brain, nicotine significantly improves fatigue (23) and depression symptoms (24,25), which are common (prevalence >50%),

quality-of-life-altering disease manifestations in sarcoidosis patients (16,26). Based upon these observations, and our supportive preliminary data, we hypothesize that nicotine will be an effective therapy for sarcoidosis. If shown to be effective, the contribution is significant because nicotine is already FDA approved for this application (established for our pilot study), is readily available to patients, is shown to be well-tolerated, and represents a low-cost, low-risk alternative to currently available treatments. As such, this project represents an opportunity to **"repurpose"** nicotine for a new therapeutic application.

C. Study Objectives

Rationale for the proposed study aims: It should first be emphasized that this Clinical Trial Pilot Study is designed to provide preliminary clinical efficacy and safety data for the purpose of planning a subsequent definitive multi-institutional clinical trial

In terms of meaningful clinical endpoints, serial pulmonary function testing, particularly forced vital capacity (FVC) is the current objective standard for assessing disease status in patients with pulmonary sarcoidosis. Thus, changes in FVC will be considered as the primary clinical endpoint for this clinical trial. However, serial FVC measurements can vary up to 10%, and expected changes in FVC with effective therapies is typically less than 5%, necessitating larger clinical trials to demonstrate efficacy. In contrast, changes in radiographic pulmonary disease manifestations in the context of effective therapies for sarcoidosis are much greater in magnitude than FVC (27). Furthermore, changes in pulmonary radiographic disease burden are an accepted surrogate for FVC and other clinical endpoints (28-31). In this regard, we have developed an objective computerized CT image analysis tool that can detect the common manifestations of pulmonary sarcoidosis and correlates strongly with FVC (32). Of note, our new unpublished data demonstrates strong correlations between the Lung Texture Score (LTS) derived from computerized CT image analysis and lung disease severity (as reflected by FVC, total lung capacity (TLC), and lung diffusing capacity) in other interstitial lung diseases (data not shown), which further validates this novel lung CT image analysis approach. Other meaningful clinical endpoints relating to disease-specific symptoms will be considered in Aim 3. For instance, fatigue is among the most common and disabling symptoms associated with sarcoidosis, which is often refractory to conventional sarcoidosis treatments (16, 26), and there is reason to believe that nicotine treatment will attenuate these symptoms (23-25). Finally, and in the spirit of a Clinical Trial Pilot Study, Aim 4 will address drug safety and tolerance, for the purpose of planning a larger, more definitive clinical trial.

Aim 1: Estimate study size for a definitive randomized control trial (RCT) based upon change in Forced Vital Capacity (FVC) in pulmonary sarcoidosis patients treated with transdermal nicotine for 24 weeks.

Aim 2: Determine if nicotine treatment is associated with improvement in radiographic lung disease burden in patients with pulmonary sarcoidosis.

Aim 3: Consider alternative clinical endpoints for future nicotine RCT based upon subjective clinical response to nicotine treatment in pulmonary sarcoidosis patients.

Aim 4: Determine the safety and tolerance of nicotine treatment in patients with pulmonary sarcoidosis for the design of subsequent definitive double-blinded RCT.

D. Investigational Agent

D1. Preclinical Data from the Pilot Study: "Nicotine for the Treatment of Pulmonary Sarcoidosis": Our pilot study sought to determine if nicotine was well-tolerated and would restore antigen-mediated immune responses in patients with active pulmonary sarcoidosis. Nicotine was reviewed as an investigational new drug (IND) by FDA and by our local IRBs to treat sarcoidosis patients. The findings of the study are detailed in our recent publication (11). To summarize, there were no serious adverse events or signs of addiction or dependency. In keeping with previous nicotine patch studies (33), minor side effects occurring at a frequency exceeding 10% were: headaches, abnormal dreams, agitation, insomnia, and local skin irritation, which improved or resolved within weeks of treatment. There was a trend towards lower fatigue scores, but no statistically significant changes were observed in reported sarcoidosis symptoms (SHQ, FAS) or measured FVC following nicotine treatment.

Nicotine treatment was associated with normalization of peripheral blood T cell subsets. In keeping with previous studies (34), active pulmonary sarcoidosis was associated with peripheral lymphopenia, including reduced FoxP3+ regulatory T cells (Tregs). Nicotine was shown to reverse peripheral lymphopenia and restore Treg populations, particularly a $CD25^-$ subset that was recently shown to be a "pre-activated" Treg. The $CD4+CD25^-FoxP3+$ Treg subtype is interesting in that it lacks suppressive actions on effector (CD4+) T cells, becoming regulatory upon stimulation (e.g., IL-2) (35). Thus, these cells are believed to be in transit to diseased tissues whereupon they adopt suppressive activity (36).

Nicotine treatment reverses peripheral anergy. Peripheral anergy to certain antigens is a well-known feature of active pulmonary sarcoidosis, and correlates with disease severity (37). In keeping with previous studies (38,39), our data shows that patients with active pulmonary sarcoidosis are specifically anergic to TLR2, TLR4 and TLR9 ligands. Moreover, nicotine treatment restored immune responsiveness to these ligands. This data is conforms to the hypothesis that sarcoidosis is associated with blunted antigen-mediated immune responses leading to impaired antigen clearance and sustained granulomatous inflammation (40). The proposed ancillary studies will determine the mechanisms by which nicotine, and more specifically α7-nicotinic receptors, restores antigen-mediated T cell responses.

Nicotine treatment did not promote tachyphylaxis. It is interesting to note that α7-nicotinic receptor expression is dramatically increased in patients with active pulmonary sarcoidosis, which is predictive of response to nicotine (11). One concern relating to the chronic effects of nicotine was the potential development of tachyphylaxis (e.g., reduced α7-nicotinic receptor expression). No change in α7-nicotinic receptor expression on immune cells was observed in the nicotine treatment group (11).

D2. Clinical Data to Date

Our previous pilot study was not powered to assess clinical endpoints relating to efficacy. However, nicotine was well-tolerated in patients with pulmonary sarcoidosis. Common (>10%) minor side effects observed in our pilot study (11), and reported elsewhere (33), includes local skin irritation, headaches, nightmares, sleep disturbances, which tend to subside with ongoing therapy. There were no serious adverse effects.

D3. Dose Rationale and Risk/Benefits

Cigarette smokers, and smokeless tobacco users, have ~2-fold reduction in risk of developing sarcoidosis (2-4). Transdermal nicotine patch delivery systems are designed to attain *in vivo* drug levels approximating that of regular cigarette smokers, which is in the 10-40 nM range (41,42). Nicotine in this concentration, when achieved in animals, is shown to suppress T-cell mediated immune responses (43, 44). The 21 mg/day nicotine patch achieves drug levels within this range humans (41,42), and is shown to be well tolerated in terms of serious adverse events in large clinical studies (45). Furthermore, the transdermal nicotine delivery

approach reduces the risk of developing dependency by providing steady nicotine levels *in vivo* (46). To the extent that nicotine levels attained in smokers prevents the development and progression of sarcoidosis, the 21 mg/day transdermal nicotine dose achieves our goal of attaining a biologically relevant dose of nicotine that will be well-tolerated in most subjects.

E. Study Design

E1. Overview of Design Summary

This will be a prospective, randomized, parallel design with multiple measures, double-blinded, placebo-controlled study conducted on adults with confirmed sarcoidosis (e.g., biopsy proven, or a clinical diagnosis made by sarcoidosis experts) sarcoidosis to determine the effects of transdermal nicotine treatment in patients with active pulmonary disease despite conventional therapies. Patients will be enrolled sequentially and based solely on eligibility. Patients will be randomized in a 1:1 ratio to receive transdermal nicotine 21 mg/daily or an identical-appearing placebo patch. Baseline measurements will be made at the screening and baseline visits on all patients prior to receiving their randomized assigned treatment to a nicotine treatment group (n = 25 patients) and a control group (n = 25 patients) assigned to placebo patch treatment. Nicotine treatment will begin with a 2 week phase in during which the dose of nicotine will be increased weekly towards the highest tolerated dose, using the transdermal drug delivery approach (7 mg patch, 14 mg patch, 21 mg patch). Patients will be maintained on the highest tolerated dose (7-21 mg patch) for the duration of the 24 week treatment protocol. Nicotine treatment will then be deescalated weekly (i.e., 14 mg, 7 mg, and then discontinued).

E2. Subject Selection and Withdrawal

Inclusion and Exclusion Criteria:

Adult male and female subjects ≥ 18 to ≤ 75 years of age will be screened for eligibility. Eligible adult patients will have:

- Strong clinical evidence of sarcoidosis confirmed by the expert pulmonologists (site PIs), diagnosed at least 2 months before screening, with evidence of parenchymal disease on a recent chest radiograph

performed as part of routine clinical care or during initial screening for this study (for the purpose of research).

- A Medical Research Council dyspnea score (47) of at least grade 1
- Patients must be on no treatment or on a stable treatment regimen for sarcoidosis for at least one month prior to enrollment in the study.

Major exclusion criteria include:

- Recent (within 3 mos) treatment with an anti-TNFalpha therapy (infliximab, adalimumab, etanercept, etc).
- Active tobacco smoking or use of smokeless tobacco products containing nicotine
- Active cardiac or central nervous system disease
- History of adverse reaction to nicotine or nicotine-containing products
- Patients with extensive irreversible pulmonary fibrosis (based upon lung biopsy or high resolution CT scan criterion)
- Inability to provide consent.
- The subject will be excluded if they have recently smoked (within 6 months) or have a diagnosis of other significant respiratory disorder other than sarcoidosis that in the opinion of the investigator would complicate the evaluation of response to treatment.
- History of substance abuse (drugs or alcohol) within 3 years prior to screening or other circumstances (e.g., psychiatric disease) that could interfere with the subject's adherence to protocol requirements or increase their risk of drug (nicotine) dependence.
- Patients with a diagnosis of current or recently active cancer (within 1 year) will be excluded.
- Pregnant women will be excluded

Ethical Considerations: Enrollment will be restricted to adults who are capable of providing informed consent and will be unbiased to race, gender or economic status. To avoid any risk of coercion, we will not recruit prisoners.

Subject Recruitment Plans and Consent Process: Patients will be enrolled sequentially and based solely on eligibility. Consent will be obtained by experienced research coordinators under the direct supervision of the site PI at each clinical site and only after obtaining written, informed consent. Subjects who are successfully screened will be assigned a subject number.

We will leverage existing CTSA (NIH) supported resources. For instance, we will utilize OSU's CTSA-sponsored "Research Match" program, a secure volunteer registry for prospective study participants, to screen

for potential study subjects and subjects will be able to actively seek the study using the "StudySearch" tool that lists research studies and clinical trials at The Ohio State University that are actively seeking participants. Additional advertising of the study will be available by entering the study into the ClinicalTrials.gov registry.

If, during the screening phase, the subject has a clinically significant worsening of sarcoidosis requiring adjustment of baseline medications, the subject will be considered a screen failure and is not eligible for randomization at that time. In this case, the subject may be rescreened after appropriate treatment has been given, and the subject has been on a stable dose of the medication for at least 4 weeks.

An informed consent form will be prepared according to the institutional requirements for informed consent and the applicable regulations. A sample consent form from the related pilot study (11) is provided in the Appendix. The appropriate IRB must approve the protocol and informed consent documents, agree to monitor the conduct of the study, and agree to review study progress periodically, at intervals not to exceed 1 year. To this end, the two Clinical and Translational Science Award (CTSA) institutions engaging in this study from Ohio (Ohio State University and Cleveland Clinic Foundation) have established a statewide collaborative agreement allowing a single IRB, in this case Ohio State University's, to assume IRB responsibilities on behalf of multiple institutions when conducting multicenter studies. This collaborative arrangement will serve to accelerate research by streamlining human subject protection processes.

The consent form will be reviewed with the prospective study subject or his/her legal representative, and the investigator will be available to answer questions regarding procedures, risks, and alternatives. The principal investigator or his/her entitled designee (as defined on the Delegation List) will obtain written informed consent from each subject or from the subject's legal representative or designee. Consent will be obtained before any protocol-specific procedures are performed. Documentation of the subject's informed consent for and participation in this study will be noted in the subject's medical record. If the subject is enrolled in the ancillary study relating to this protocol, a sub study-specific consent must also be used. The subject or his/her legal representative must be provided with a copy of the consent form for the main study and a copy of any separate consent form for the sub study (if applicable).

Randomization Method and Blinding: Patients will be randomized to nicotine or placebo in a 1:1 ratio. Randomization will be within each site (balanced by site) and use permuted blocks of varying size (2,4,6). The randomization scheme will be developed by the study statisticians and the scheme allocated through the blinded study subject number. Study drug will be pre-packaged with an assigned study subject number, corresponding to the allocated randomized treatment. All baseline measurements will be obtained at the screening and baseline visit before allocation of the random assignment. Identically labeled nicotine and placebo patches will be randomly designated treatments "A" or "B", and will be distributed by each clinical center.

Risks and Benefits: Nicotine intolerance rates are very low, even at doses 2-fold greater than the maximum dose used in this study (48). Common (>10%) minor side effects observed in our pilot study (11), and reported elsewhere (33), includes local skin irritation, headaches, nightmares, sleep disturbances. We are aware of the potential for serious adverse events, particularly cardiac arrhythmias (49) and seizures (50) in patients with underlying heart or central nervous system disease, respectively, and will exclude patients with these risk factors from enrollment in this study.

Early Withdrawal of Subjects: Patients will be immediately withdrawn from the clinical trial for any of the following reasons: 1) at the request of the subject, 2) at the discretion of clinicians caring for the patient, 3) as

advised by the DSMB, 4) if the subject is unable to comply with the study protocol. The Research Manager will be notified within 48 hrs of the subject's withdrawal and will record the reason for withdrawal.

Data Collection and Follow-up for Withdrawn Subjects: Withdrawn subjects will be encouraged to return for the post intervention study-related procedures, including image analyses, surveys, and PFTs, in order to include them in analysis, following the intention-to-treat principle.

E3. Study Drug

Description: Sustained release transdermal nicotine (7, 14 and 21 mg daily dose patches) and matching placebo patches will be provided. The nicotine patches and matching control patches will be administered daily according to the manufacturer's recommendations and in compliance with a FDA approved dosing regimen.

Treatment Regimen: To optimize patient compliance and minimize side-effects, nicotine (or matching placebo patch) treatment will be initiated at 7 mg/day and escalated at weekly intervals to 14 mg/day, then 21 mg/day, as tolerated. If intolerance develops, the dose will be reduced to the highest tolerated dose. The dose will be maintained at 21 mg/day (or the highest tolerated dose) for 24 weeks, at which time the dose will be deescalated to 14 mg/day, then 7 mg/day at weekly intervals, after which nicotine treatment will be discontinued.

Method for Assigning Subjects to Treatment Groups: Patients will be randomized to nicotine or placebo patches in a 1:1 ratio. The randomization scheme will be developed by the study statisticians and the scheme allocated through the blinded study subject number. Study drug will be pre-packaged with an assigned study subject number, corresponding to the allocated randomized treatment. Treatment allocations will be blinded to study clinical staff, treating clinicians and patients. Nicotine and replica placebo patches will be randomly designated treatments "A" or "B", and will be distributed by each clinical center. All baseline measurements will be obtained at the screening and baseline visit before allocation of the random assignment

Preparation and Administration of Study Drug: Patients will be instructed to apply a new patch to their skin every day. The site should be clean (e.g., with warm soapy water) and dry at the time of application and only one patch should be placed at a time (old patches must be removed before applying a new one). The backing of the patch is removed to reveal the sticky surface, which is placed on the skin. To avoid local skin irritation, a new site is recommended (e.g., on the upper arm) each day.

Subject Compliance Monitoring: Subject compliance with nicotine will be monitored by history and objectively at regular intervals, as reflected by measuring nicotine and cotinine, a stable nicotine metabolite, as per the Study Schedule of Events (Table 1), described below.

Prior and Concomitant Therapy: As described in the Inclusion Criteria, above, study subjects will remain on prior and concomitant medications (if applicable), as tolerated, for the duration of the study. The addition of new medications or changes in current medication doses will be considered a violation of the study protocol. However, the patients will be retained in the study to consider trends in de-escalation or escalation of treatment in the treatment and placebo groups.

Packaging and Blinding: The packaging of the nicotine patches and placebo patches will be identical in appearance such that the patients and research staff remain blinded to treatment. Identically labeled nicotine and placebo patches will be randomly designated treatments "A" or "B", and will be distributed by each clinical center.

F. Study Procedures

F1. Screening Procedures

This study will be conducted in accordance with current US FDA regulations and guidelines, ICH guidelines on GCP, the principles of the Declaration of Helsinki, as well as all other applicable national and local laws and regulations. Patients will be screened by fully qualified research coordinators at each clinical center based upon the inclusion and exclusion criteria. Verification of active pulmonary sarcoidosis will include review of pathology reports and/or tissue samples (if available), and review of radiographs (chest x-ray and/or lung CT scans) by a board-certified pulmonologists (i.e., the site PIs). A chest x-ray will be performed if the patient has not had one done within the past year. A thorough history and physical exam will be conducted and an ECG will be routinely obtained (if not already available) to screen for cardiac disease and other exclusion criteria, as described above.

F2. Schedule of Measurements

The schedule for study visits including the assessments and procedures to be performed at each visit is presented in the Schedule of Events (see Table 1). The scheduled study visits should occur at the specified week post-randomization ± 7 days unless otherwise specified. It is encouraged that study visits be scheduled in the morning, at the same time for each visit, unless extenuating circumstances exist. In addition to scheduled visits, monthly telephone interviews will be conducted by the study site research coordinators to establish/encourage compliance with nicotine (placebo) patches and to screen for adverse events.

F3. Safety and Adverse Events

Safety analyses will be based on treatment received. Safety data, including but not limited to, AEs, serious AEs (SAEs), infections, serious infections, mortality, changes in laboratory assessments, and changes in vital signs. Treatment-emergent AEs will be summarized by treatment group.

All untoward events occurring between the time of obtaining informed consent through Week 34 must be reported as an AE. In keeping with the standards of clinical trials research, the relationship of AEs to nicotine will be classified as “definitely related”, “possibly related” or “unrelated”, and we will further document the reason for subject dropout, report subjects with multiple adverse events, and track disease exacerbations (requiring escalation of corticosteroid dose or unscheduled healthcare visits).

The severity of AEs will be made using the following general categorical descriptors:

- **Mild:** Awareness of symptoms that are easily tolerated causing minimal discomfort and not interfering with everyday activities.
- **Moderate**: Sufficient discomfort is present to cause interference with normal activity.
- **Severe**: Extreme distress causing significant impairment of functioning or incapacitation and preventing normal everyday activities.

Based upon our pilot study (11) and previous published reports (33), we expect to see an increase in the frequency of mild side effects with no significant SAEs in the nicotine treatment group compared to placebo controls. These minor side effects tend to abate with continued use of nicotine, and only one subject withdrew from the pilot study due to local skin irritation at the site of the patch. For this reason, we will encourage the subjects to alter the site of the transdermal patch on a daily basis.

There is a potential for a placebo effect with respect to the use of a patch in the nicotine treatment group compared to controls. However, a placebo effect would be expected to influence subjective measures (e.g., dyspnea scores); whereas, objectives measures of lung function or radiographic findings would be effected very little, if at all.

Subjects will be instructed to call the investigator if their symptoms of sarcoidosis worsen. Based upon the investigator's assessment of the subject's symptoms, functional parameters, and the subjects' history, a clinic visit may be scheduled. If in the investigator's opinion the symptoms of sarcoidosis have worsened sufficiently to require treatment, the investigator will assess the severity and initiate appropriate treatment depending upon the subject's condition. Despite escalation of treatment, the patient will remain enrolled in the study.

Patients will be followed for an additional 4 weeks after nicotine treatment to determine if they required any change in medications, including escalation of therapy for sarcoidosis, and to screen for signs of nicotine dependency or withdrawal. Although nicotine dependency did not occur in the pilot study and is documented to be rare in the setting of transdermal preparations (33), likely due to steady and sustained drug levels (45,46), it remains a potential concern. As such, study subjects demonstrating nicotine dependency or addiction symptoms (e.g., nicotine craving or inability to discontinue nicotine-containing products) one month after discontinuation of the drug will be provided support to engage in a smoking cessation program, which has extensive experienced with nicotine dependency and addiction management (51).

F4. Study Outcome Measurements and Ascertainment

The primary endpoint of the study will be the change in FVC, measured before and after treatment with nicotine or placebo for a total of 24 weeks.

The secondary clinical endpoint of major interest will be the change in total burden of lung disease, as reflected by the computer-generated CT image analysis or changes in lung function. Data files will be de-identified and thereby blinded to the investigative team. Moreover, the computer-generated Lung Texture Score (LTS) ascertained from these analyses are objective, highly reproducible, and strongly correlate with standard physiological (PFT) measures (32).

Other secondary endpoints will determine if changes in LTS correlate with changes in simultaneous PFT measures or measures of the rate of nicotine metabolism, as reflected by the ratio of 3-hydroxycotinine to cotinine in serum samples. Finally, the effects of nicotine treatment on disease-related symptoms will be determined by comparing the results of surveys (SAT, SGRQ, FAS) conducted at regular intervals during the study protocol. Again, these analyses will remain blinded to treatment allocation until the close and un-blinding of the study.

PFT measurements:

The post-bronchodilator FVC, forced expiratory volume in 1 second (FEV1), peak expiratory flow rate (PEFR), and forced expiratory flow at 25% to 75% of vital capacity (FEF25-75) will be measured according to the body temperature, pressure, and saturation (BTPS) standard convention. PFTs will be repeated up to 8 times to obtain 3 acceptable readings according to American Thoracic Society (ATS) guidelines. Acceptable repeatability is achieved when the difference between the largest and the next largest FVC is ≤ 0.150 L and the difference between the largest and the next largest FEV1 is ≤ 0.150 L. However, if a subject is too tired to meet this consistency requirement, the PFT values from the best effort can still be accepted as long as this best effort meets the other ATS criteria. Subjects should refrain from using short acting bronchodilators for at least 4

hours and long-acting bronchodilators for at least 12 hours prior to the screening visit. Percent predicted FVC and FEV1 will be calculated according to the Crapo equation (52), with correction for race.

CT scans and x-ray: CT scans will be performed at the baseline assessment or within 3 months prior to enrollment so long as the following criteria are also met: 1) the CT scan was obtained for clinical reasons and on the same scanner used for the research study, 2) the patient's sarcoidosis treatment regimen was unchanged within the past 3 months, and 3) respiratory symptoms (cough, dyspnea) were stable. The imaging protocol will be standardized across the clinical centers: Non-contrast CT scans will be obtained by using a helical technique with a 16- or 64-detector row CT scanner. Images will be obtained from lung apices to the lung bases in a single breath hold with the following parameters: collimation, 1.25 or 0.625 mm; field of view, 36 cm; beam pitch, 1.35 or 1.375; gantry speed, 0.5 or 0.6 sec/rotation; 120 kVp; 150–200 mA. The image files will be sent from a proxy PACS server located at each clinical center and transferred via a Virtual Private Network (to assure data confidentiality) and processed by Dr. Erdal via a workstation located within the Department of Radiology at OSU. The image data will be reformatted with a 5-mm section thickness using standard lung algorithms. The lung image files are then segmented using Houndsfield units (-200 to -1000), and a two-point correlation function based approach to determine the presence of texture mismatches representing diseased lung is conducted within each volume of lung tissue using a proprietary plug-in program that can be operated on the publicly available NIH Image J Toolkit (http://rsbweb.nih.gov/ij/index.html) to create a regional lung texture score (LTS). The LTS for a given volume is integrated into an LTS for the entire lung, reflecting the overall burden of lung disease (32).

A chest x-ray will be performed at screening if one has not been done within the past year.

Prior to all radiation procedures, a urine pregnancy test will be done. If the result of the urine pregnancy test is positive, a serum pregnancy test will be done. P

Blood draws: Study subjects will undergo scheduled venopuncture under sterile technique for the purpose of obtaining 10 ml of blood to measure nicotine and nicotine metabolites (at baseline, 10 and 28 weeks). The blood samples will be labelled with the same identifier used for the subject's web-based data entry (REDCap), and will be stored on site at OSU and CCF at -80º F in a secure (password protected) laboratory until the final analysis for nicotine and related metabolites (cotinine). The samples from CCF will be shipped by same-day delivery on dry ice (i.e., -80 º F) as a single batched sample prior to analysis.

Surveys: Well-standardized surveys will be administered according to the Study Schedule of Events (Table 1). These will be conducted during scheduled study-related visits or by mail (self-addressed first class mail through the US postal service). The following standardized surveys will be employed (examples of each are provided in the Appendix):

The **Fatigue Assessment Scale** (FAS) is a 10-item patient self-report instrument with five items reflecting physical fatigue and five assessing mental fatigue. The response options range from never (1) to always (5) for a total score from 10 to 50. Psychometric qualities are reported as satisfactory by the authors, and the instrument has been able to differentiate healthy respondents from patients with sarcoidosis (53,54).

The **St George's Respiratory Questionnaire** (SGRQ) is a disease-specific survey instrument designed for use with adult subjects to assess the impact of respiratory disease and its treatment on the subject's health outcomes (55). The SGRQ is self-administered and is usually completed within 10 to 20 minutes. The instrument contains 76 items in three domains: symptoms (frequency and severity), activity limitations, and impacts on social and psychological functioning. The response categories use a Likert scale or dichotomous

responses. The methods used for development of the instrument have not been published and criteria for item selection are unclear. The psychometric properties have been reported to be adequate. The instrument has been used extensively in research with asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, IPF, and interstitial lung disease.

The **Sarcoidosis Assessment Tool** (SAT) is a self-administered health-related quality-of-life (HRQL) instrument that is validated against other standardized HRQL instruments for patients with active sarcoidosis and is further validated to specifically detect changes in the severity of lung involvement (56).

G. Statistical Plan

G1. Sample Size Determination and Power

Depending on the clinical efficacy of nicotine compared to another immune modulating agent (infliximab), we expect to see up to 3% improvement in FVC in the nicotine treatment group based upon a comparable study design in patients with active pulmonary sarcoidosis (27). With the expected 25 patients per group and average change variance at a minimum of 3% for FVC, the precision of a comparison of means is roughly adequate for planning, but is insufficient for achieving significance unless the nicotine effect on FVC is large (e.g., >5%) as compared to controls. We will report *p*-values for these as secondary outcomes, and derive meaning only if our primary outcome (FVC) shows significance, i.e. we will use some of the logic of gatekeeping hypothesis testing (57). Assuming 5-10% variance (standard deviation) between consecutive FVC measurements, and a 3% change in FVC with treatment the estimated study size would range from 44-178 in each arm of the subsequent Phase II trial.

Our pilot study was not designed to detect radiographic changes after nicotine treatment (11). However, based upon the results of a recent Phase II sarcoidosis clinical trial structured almost identically to the study proposed herein (total **n = 40/group**), wherein the radiologist-generated “radiology score” (R-score) change from baseline was >30% post-treatment with low dose (3 mg/kg) anti-TNFα drug (i.e., 3.11 to 2.07, on a scale from 0-4 with “4” being most severe disease, $p < 0.001$) and changed significantly relative to matching controls [i.e., net change -1.33 (+0.29 ± 2.21 for controls and -1.04 ± 2.02 for treatment group, $p < 0.001$)] (27), we predict that **55 patients** in each group (nicotine treatment and control) will be sufficient to detect a 15% difference in LTS score change before and after treatment with 90% power and type I error at 0.05 assuming a standard deviation of LTS score change at 20%. We consider the latter to be a conservative assumption based upon previous texture-based computerized volumetric CT analysis studies (as will be employed for this study) designed to detect changes in lung nodules of varying sizes over time wherein the standard deviation in the volumetric score was less than 10% across a broad range of nodule sizes and textures, including solid and ground glass densities (which are very common in sarcoidosis patients) (57). Of note, if the standard deviation of the LTS is closer to 10%, this study size will be sufficient to detect a 5-10% change in LTS with a power of ~42-94%. Thus, this pilot study may underpowered to detect a significant change in the LTS following nicotine treatment, but will be useful for estimating the study size required for a subsequent adequately powered Phase II trial.

G2. Interim Monitoring and Early Stopping

As above, we conservatively predict that nicotine treatment will result in a 3% and 10% reduction in FVC and LTS, respectively, relative to the control group. In the spirit of a pilot study, we are unsure of the potency of nicotine in terms of preventing progression or promoting regression of disease, as reflected by the LTS.

Moreover, there is a possibility that nicotine treatment could be ineffective or associated with serious side-effects in too many patients. As such, the DSMC will continuously monitor the progress of the study and will consider early discontinuation of the trial if nicotine treatment is associated with excess serious adverse events (SAE) (arrhythmias, seizures, syncope, new cancers, death).

G3. Analysis Plan

The research data for each Aim will be analyzed at the time of unblinding (year 03). The exception being DSMC data analysis for study related AEs and SAEs, and for analyses relating to performance monitoring, as detailed below.

G4. Statistical Methods

The primary study endpoint will determine if nicotine treatment improves an objective measure of lung function that is commonly used in sarcoidosis clinical trials, and is considered to be the "gold standard" clinical endpoint for assessing clinical response in the setting of pulmonary disease activity (59). We will use a longitudinal model, which has certain advantages over the usual ANCOVA (using baseline LTS as covariate) (60). In this analysis both baseline and and post intervention LTS are dependent variables in the longitudinal model (60). Using SAS's Proc Mixed (61), all patients will be included in analysis, whether they are missing either a baseline (unlikely to happen) or a post intervention measurement. Included in the fixed effects model will be race and gender. Interactions with either will only be explored in a sensitivity analysis. With just two measures, we will use a saturated covariance structure (which allows the variances of the baseline and post measures to differ).

For the LTS scores, a longitudinal model, as described in Aim 1, will be used to compare LTS results in each treatment group at baseline and at the completion of the 24 week treatment protocol. Correlation between FVC and LTS: Bivariate plots will be used to describe the relationship between changes in FVC (Aim 1) and LTS (Aim 2). With 25 patients in the nicotine group, where substantial correlation of changes is expected, the standard error of the correlations will be ~0.15 for moderate values.

G5. Missing Outcome Data

Assuming a 10% dropout before follow-up, complete data will be obtained from ~22 subjects in each arm of the study. The missing at random (MAR) assumption will be our primary adjustment for potential missing data bias (62,63). Sensitivity analyses will confirm the main findings as recommended by the special NAS panel on missing data in clinical trials (63).

G6. Unblinding Procedures

Under normal circumstances, so as to not compromise the integrity of the study, the blind should be maintained. Otherwise, the blind should be broken only if specific emergency treatment would be dictated by knowing the treatment status of the subject. It is recommended that the investigator contact a designee of the DSMB to discuss the situation. Telephone contact with the DSMB representative will be available 7 days per week. However, if the investigator is unable to contact the DSMB, or emergency unblinding is considered medically necessary, the investigator may determine the identity of the treatment by contacting The Ohio State University's Clinical Trials Office. Subjects who have had their treatment assignment unblinded will still be expected to return for scheduled evaluations. The date, time, and reason for the unblinding must be documented in the source documents.

H. Handling and Security

H1. Confidentiality and Security

Subject information collected in this study will comply with the standards for protection of privacy of individually identifiable health information as promulgated by applicable local/regional/national requirements for subject confidentiality (e.g., Health Insurance Portability and Accountability Act as mandated in Title 45 CFR, Parts 160 and 164). All records will be kept confidential and the subject's name will not be released at any time.

Subject records will not be released to anyone other than the study personnel or its designee(s), and responsible government agencies, when requested. In all cases, caution will be exercised to assure the subject's confidentiality. The developed REDCap database used for data collection will be stored on a password protected, firewall protected server maintained by OSU's CTSA.

H2. Training

Initial training for core and study-specific elements will be conducted during week two following a review of the protocol by the DSMB. Core elements include 1) recruitment; 2) eligibility and screening; 3) informed consent; 4) enrollment; 5) protocol implementation, 6) data collection, procedures and forms completion; 7) data entry and management and 8) adverse event monitoring and reporting. Follow-up training will occur during site visits and by conference calls as needed. Staff certification is based on full attendance at initial central training at OSU (week 1) and task observation during subsequent site visits. Site visits will occur four times per site during the study period.

H3. Case Report Forms and Source Documents

All subject data, including case report forms and source documents, will be uploaded to a password protected web-server maintained by The Ohio State University's CTSA.

H4. Records Retention

OSU and CCF will maintain the records of drug disposition, worksheets, and all other study-specific documentation for at least 3 years after the completion of the study.

H5. Performance Monitoring

We will define a set of comprehensive data quality assurance and programmatic and/or project-specific tracking metrics. Examples of such metrics include: data completion levels, timeliness of data entry, conformance of data with protocol-specified range and value checking logic, as well as the satisfaction of protocol-driven milestones such as participant and bio-specimen accrual rates. We will use an instance of the Jasper Business Intelligence Suite (JBIS) to generate and visualize such metrics in both standard scheduled reports (delivered electronically via e-mail transmissions and a secure web portal interface) and a real-time dashboard, again delivered via a secure web portal. All metrics will be reviewed on at least a quarterly basis by the program's leadership to ensure their adequacy for data monitoring and project management/planning purposes.

I. Study Monitoring, Auditing, and Inspection

The first visit is mandated to occur after the 2nd and before the 5th enrolled study subjects to assure that adequate training has occurred and appropriate local documentation is in place including eligibility evaluation,

informed consent, data collection, entry and management, and specimen collection and processing. Each site visit will also involve audits of a small random sample of participant records. The first site visit will also assure that the sites have adequate plans for recruitment. Site visit team will subsist of a designated OSU clinical coordinator and will include Dr. Crouser at least once a year. Prior to the visit, the OSU clinical coordinator will review performance reports to identify potential areas of concern. OSU will work with the Cleveland Clinic to plan the visit agendas to best serve the projects' needs for problem-solving, monitoring, and observation of tasks. The site visit team will also use the visit to obtain information regarding what additional database tools or resources are needed for efficient functioning at the sites. The site visit team will submit a report to the CC and provide a two week interval to respond. The report and response will then be submitted to the Steering Committee. If needed, performance improvement plans will be developed and OSU's CTSA will assist clinic staff in implementing and monitoring subsequent performance. Data monitoring will be informed by quarterly review of JBIS generated metrics as previously described.

A Data and Safety Monitoring Board (DSMB) will be appointed to oversee study design, implementation, patient recruitment, interim analyses, adverse events and data management. The DSMB will be provided with routine reports on recruitment, timeliness and quality of data collection, adherence to protocol, and adverse effects for each of their regular meetings. Serious adverse events will be reported to the DSMB within 48 hrs. Details of the DSMB structure and function are as follows:
The specific responsibilities of DMSB will include the following:

1. Review the research protocol, informed consent documents, and plans for data and safety monitoring;

2. Evaluate the progress of the trial, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the trial sites, and other factors that can affect study outcome;

3. Consider factors external to the study that may be relevant, such as scientific or therapeutic developments which could have an impact on the safety of the participants or the ethics of the study;

4. Review study coordinating center performance, make recommendations, and assist in the resolution of problems as reported by the PI;

5. Protect the safety of the study participants and report on the safety and progress of the trial;

6. Make recommendations to the research team and sponsor, as required, concerning continuation, termination or other modification(s) to the trial;

7. Ensure the confidentiality of the trial data and the results of monitoring;

8. Assist on any problems with study conduct, enrollment, sample size and/or data collection.

9. Review data for accuracy and quality

The DSMB will be composed of pulmonologists experienced with clinical research [Marc Judson, MD-Chairperson, and Kenneth Knox, MD] a pharmacologist and biomedical scientist [Daren Knoell, PhD], a biostatistician [Lai Wei, PhD], and a research subject advocate/ethicist [To be named]. All members are independent of the research team as well as have no financial, scientific or other conflict of interest with the project. The Chairperson will serve as the primary contact between the research team and DSMB. The DSMB in conjunction with the research team, shall meet before the initiation of the study, i.e. prior to subject enrollment, and at 12 month intervals throughout the duration of the project until after the final subject has completed all study-related assessments but no sooner than the time point at which study-related adverse

events would likely present. All adverse events will be followed until resolution as is feasibly possible, inclusive of those events that result in premature withdrawal from the study.

The DSMB meetings will include both an open and closed portion of review. An emergency meeting may be called at any time by the request to the Chairperson and/or the Principal Investigator. Beyond the interim analysis as described above no other formal stopping rules will be employed. Study progress reports will be prepared by the research team and distributed to the DSMB prior to the scheduled meeting(s). These reports will include updates on the protocol inclusive of revisions, enrollment progress and projections, retention, and safety data inclusive of description, severity, attribution, response, reporting and resolution of those events as well as if any events are unanticipated. Serious and/or unanticipated event(s) deemed to be related to the procedures of the study will be brought to the attention of the DSMB and then subsequently to the sponsor and IRB(s), as appropriate and as soon as possible. Moreover, in the event that any proposed change to the study may elevate the risk to benefit ratio such requested changes would be brought to the attention of the sponsor Program Officer, as per sponsor policy, prior to the implementation of such proposed change(s), e.g., the addition of a new study population that would be at higher risk for the existing study procedures, the addition of new procedures greater than minimal risk, any modifications to the existing study procedure(s) that may increase overall risk, and/or the addition of a new clinical study and/or study arm not originally proposed that is greater than minimal risk.

A written summation of the DSMB's review(s) of the study will be submitted to the PI. As appropriate, any such report will be forwarded to the Program Officer for the sponsor, and/or IRB(s). Continuation and/or revision of the study will be based upon the collective expertise of and guidance offered by the DSMB.

J. Study Administration

J1. Organization and Participating Centers

Two clinical centers with extensive experience in sarcoidosis-related clinical trials, and representing diverse sarcoidosis populations will participate in the study. Both centers are all supported through Clinical and Translational Science Awards (CTSA) and will coordinate their efforts through the CTSA-supported Center for Clinical and Translational Science (CCTS) at each facility. The Ohio State University (OSU, Columbus, OH) will serve as the data coordinating center and will oversee subject recruitment and data quality assurance. Research subjects will also be recruited from a large sarcoidosis specialty clinic located at the Cleveland Clinic Foundation, Cleveland, Ohio. The demographics of sarcoidosis in Cleveland and Columbus are distinct in that the latter is enriched with African Americans (AA). The demographics of Columbus closely resemble that of the U.S. in general (64), and AAs in the U.S. share common ancestry (65). In total, *these two centers actively manage ~ **2000** sarcoidosis patients, most of whom have pulmonary involvement* (see Table 2). And each center has successfully recruited to NIH-supported clinical trials relating specifically to lung diseases (Table 3).

J2. Committees

A Steering Committee (SC) comprised of the PIs from each clinical site, directors of administrative, protocol and clinical research will oversee patient recruitment, the implementation of the research protocols, and the generation of data towards the achievement of the proposed study aims. A Data and Safety Monitoring Board (DSMB) will be appointed to oversee study design, implementation, patient recruitment, interim analyses, adverse events and data management.

J3. Subject Stipends

To cover travel-expenses and other costs relating to participation in this project, subjects will be provided a stipend of $50 for each of the 6 scheduled protocol-related visits to cover parking/travel expenses. Additional reimbursement for travel will be available as needed.

J4. Study Timetable

Study Timeline and Milestones

Month	1	2	3	6	9	12	18	24	30	36	48
IRB approval			x								
Site Visit		x	x			x		x			
SC meeting	x		x	x		x	x	x	x	x	
Subject Recruitment				x	25%	x	50%	75%	100%		
DSMb	x						x				
External Advisory Board			x			x		x		x	
Final Data Analysis										x	
Publications											x

See Study Timeline and Milestones Table.

K. Data Sharing and Publication Plan

In order to enable public access to all project-related data sets, we will use a multi-modal strategy, including: 1) regular submission of project-generated data sets to public repositories, including those associated with the National Library of Medicine's National Center for Biotechnology Information (NCBI); 2) the deployment and configuration of one or more ProServer DAS (Distributed Annotation Service) servers to enable public access to all project-generated bimolecular data sets (his type of server complies with the standards established by the international bioinformatics community for open data access, and will be registered with a central DAS registry maintained by the Sanger Institute [http://www.dasregistry.org/]); and 3) the provision of an open-access section of the previously described (see Clinical Research Protocol) Confluence WIKI where project participants will be able to post and curate technical reports, publications, and other summary data/information concerning programmatic activities and research products. Where appropriate, all data sets and documentation generated via this project and shared through any of the preceding mechanisms will be made to conform to applicable National Technical Information Service standards and guidelines.

This study represents a joint effort of SC members (Drs. Crouser, Jackson and Culver), and as such, the parties agree that the recommendation of any party concerning abstracts, manuscripts or texts shall be taken into consideration in the preparation of final scientific documents for publication and presentation. As such, decisions relating to the validity of the data, authorship (i.e., based upon relative intellectual contribution), and other publication plans will be at the discretion of the SC.

OSU's CTSA will support the preparation of manuscripts resulting from the study-wide protocols by providing data analysis, statistical consultation, and editorial support. Manuscripts and presentations arising from the primary clinical trial will be submitted for review to the SC. Following completion of a draft manuscript, the SC will conduct a thorough manuscript review for accuracy and clarity. The SC, with assistance from OSU's CTSA staff, will track all proposed and approved publications and coordinate the entire publication process from proposal through review, analysis, preparation, publication, and dissemination

In accordance with national and local requirements, this study protocol will be listed in a publicly accessible clinical trials registry and given a unique identifier. Additionally, the results of this clinical study will be

disclosed on a publically accessible clinical trials results database, regardless of the outcome.The OSU CCTS Research Informatics Services Core will be used as a central location for data processing and management. Vanderbilt University, with collaboration from a consortium of institutional partners (including OSU) and the NIH National Center for Research Resources, has developed a software toolset and workflow methodology for electronic collection and management of research and clinical trial data. REDCap (Research Electronic Data Capture) data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the CCTS Research Informatics Services Core. As part of the data dictionary development process, individual fields can be denoted as “identifiers”. When exporting a de-identified dataset, these variables are omitted. Additionally, the data export tool also allows for the shifted of dates for a limited data set export. REDCap provides a secure, web-based application that is flexible enough to be used for a variety of types of research, provides an intuitive interface for users to enter data and has real time validation rules (with automated data type and range checks) at the time of entry. It offers easy data manipulation with audit trails and ad hoc reporting functionality for reporting, monitoring and querying patient records, and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). REDCap is 21 CRF Part 11 capable. Currently, REDCap installations support electronic signatures by positively identifying the user through a unique username and password combination. The provisioning of accounts and user access to specific database(s) is integrated with the OSU Medical Center LDAP authentication service for studies containing protected health information (PHI), and the provisioning of access and specific user rights for all studies are managed by CCTS staff.

Study Phase	Screen	Baseline	Nicotine Escalation	Stable Nicotine Treatment Phase						Nicotine Deescalation	Follow-up
Week	-4-0	0	0-2	6	10	14	18	22	26	26-28	
Consent	x										
Inclusion- Exclusion	x										
Confirm Dx	x										
Dyspnea Score	x										
CXR (if necessary)	X										
ECG	x								x		
Compliance & AEs			x	x	x	x	x	x	x		x
Vital Signs	x	x			x		x		x		
Randomize		x									
CT chest		x							x		
PFTs		x							x		
Blood		X			x				x		
Surveys		x			x		x		x		x
Medication Review	x				x		x		x		x
Dependence											x

Table 1: Study Schedule of Events

Tables

Table 3. Study Site Recruitment History

Site	Study Sponsor/Description	Project Role	Recruitment Goal	# recruited	Timeframe
*OSU	NIH/LOTT Emphysema	Site PI	21/year	72 (24/yr)	3 yrs
OSU	NIH/HIV Emphysema	PI	264	264	4 yrs
OSU	ATS-FSR/Nicotine Tx for Sarcoid Pilot	PI	15/yr	19 (15/yr)	15 months
CCF	NIH/IPFNet	Site PI	17	21	2 yrs

*Second highest recruitment among 17 participating centers

	Ohio State University	Cleveland Clinic Foundation	Total
Patient Visits (2012)	794	1198	1992
New Patient Visits	72	95	167
Female %	61	58	59
White/AA/other (%)	50/45/5	60/26/14	56/34/10

Table 2. Demographics of the sarcoidosis patient populations at participating clinical centers .

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